

Occurrence of cyanobacteria (*Richelia intracellularis*)-diatom (*Rhizosolenia hebetata*) consortium in the Palk Bay, southeast coast of India

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Symbiotic association of heterocystous cyanobacterium, *Richelia intracellularis* Schmidt with oceanic centric diatom, *Rhizosolenia hebetata* is reported from the Palk Bay, southeast coast of India. One to six trichomes of *R. intracellularis* were occluded inside the periplasmic space of *R. hebetata*, with their prominent heterocyst pointing towards the valve of the host. Each of these trichomes had 14 to 23 vegetative cells capped by a terminal heterocyst enriched with the nitrogenase enzyme. Density of *Rhizosolenia* containing *R. intracellularis* ranged between 120 and 260 cells L⁻¹, and present uniformly in the water column. *R. intracellularis* is a diazotroph, can contribute substantially to the N₂ budgets thereby, promoting a different food web in the Palk Bay.

[**Keywords:** Cyanobacterium, Diatom, Diazotrophy, Palk Bay]

Introduction

Cyanobacteria, a photosynthetic prokaryote (blue-green algae) is a wide spread planktonic organism commonly found in the rivers, rocks and soil. Significance of this diverse community is its capability to fix nitrogen (N) in the warm, sunlit waters of tropical oceans¹. It is estimated that the annual N fixation of 4.79×10^{12} g constitutes one quarter of the total input of nitrogen to the sea². Morphologically, cyanobacteria exist as coccoid, filamentous, non-heterocystous or heterocystous forms and possess the unique cells of 'Heterocysts', which can fix atmospheric nitrogen. *Trichodesmium* and *Richelia* are the most important nitrogen fixing (diazotrophic) marine cyanobacteria³. Of these, the *Trichodesmium* spp. usually found in the warm oligotrophic waters are devoid of heterocysts; whereas *Richelia intracellularis* is the only heterocystous cyanobacteria in the marine waters². Many of the cyanobacterial communities are found symbiotically (inside or outside) associated with larger phytoplankton (diatoms and dinoflagellates) in oligotrophic waters^{4,5}.

Materials and Methods

Palk Bay (PB), a shallow water body located in the southeast coast of India between Sri Lanka and Indian

sub-continent, seemed to be biologically productive than its counterpart, the Gulf of Mannar (GoM) as it receives Bay of Bengal water with lower salinity and higher nutrients⁶. Water quality monitoring in the PB and GoM was carried out in March 2010 as a part of the Environmental Impact Assessment (EIA) programme for alignment 4 A of Sethusamudram channel. Water samples were collected from the surface, mid- and bottom depths using a 5L Niskin bottles for analyzing inorganic nutrients⁷ and phytoplankton characteristics (biomass and abundance). A portable CTD (Seabird SBE 26^{plus}) was used to measure water temperature (accuracy $\pm 0.001^\circ\text{C}$) and salinity (accuracy ± 0.0001). Estimation of chlorophyll *a* was done fluorometrically⁸. Lugol's (acid) iodine fixed phytoplankton counts⁹ and identification¹⁰ were made with a stereoscope inverted microscope (Olympus CK 30).

Results and Discussion

During the identification of phytoplankton samples collected from stations A1 and A2 (Fig. 1), observed a diatom-cyanobacterium symbiotic association. Inverted microscope images showing a filamentous structure inside the host cell (*Rhizosolenia hebetata*, a ubiquitous centric diatom) was identified as *Richelia intracellularis* Schmidt, a diazotrophic marine cyanobacterium. Furthermore, there were upto 6 trichomes of

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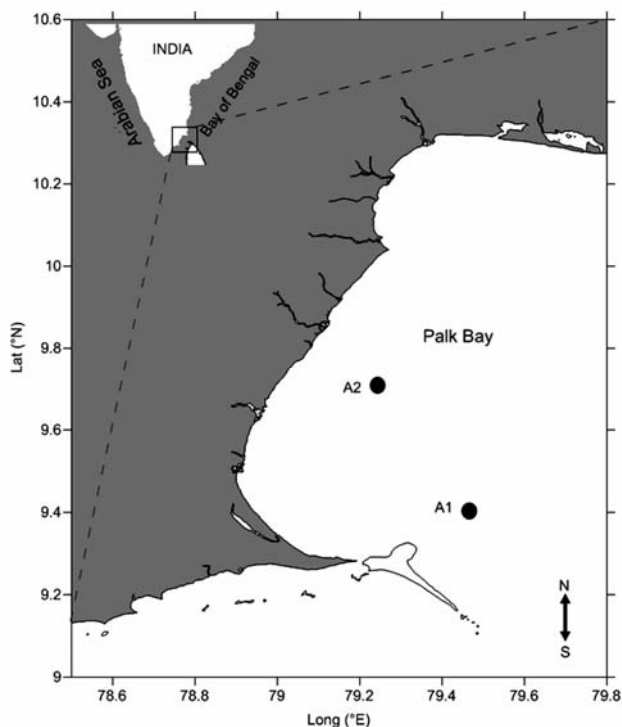


Fig. 1— Station location where diatom - cyanobacteria symbiotic association observed

R. intracellularis inside each of the host cell. Trichome was capped by a heterocyst orienting towards the valve of the host (Plate 1), whereas a single trichome was placed completely inside the valve of *R. hebetata*, and more than two trichomes of *R. intracellularis* were partially concealed or attached to the boundary of the host cell (Plate 1). Length of *R. hebetata* ranged between 65 and 79 μm and had a width of $\sim 10 \mu\text{m}$ at the centre. Each *R. intracellularis* was possessed 14 to 23 vegetative cells with dimensions ranging from 22.2 to 29.5 μm (length) and 2.4 to 2.9 μm (breadth). Abundance of *R. hebetata* containing *R. intracellularis* was ranged between 120 and 260 cells L^{-1} , and it constituted $<10\%$ of the total phytoplankton density. *R. intracellularis* is a heterocystous cyanobacterium normally seen as an endosymbiont in diatoms^{2,4} (*Rhizosolenia* sp., *Hemiaulus* sp. and *Pleurosigma* sp., etc). However, in certain diatoms like *Chaetoceros compressus* and *Bacteriastrum* sp., they live as an epiphyte, where the host consumes a part of nitrogen excreted by the *Richelia*¹¹.

Water column (surface, 5m & 10m) was relatively warmer (30.1-31.7°C) at both these locations, but salinity was relatively lower at station A2 (28.1-30.1) than at station A1 (31-32.1). Concentration of Nitrate

(NO_3) varied marginally (0.2 to 1.1 μM), but chlorophyll *a* was higher at station A2 (0.9-1.8 mg m^{-3}) as compared to station A1 (0.4-0.8 mg m^{-3}). Phytoplankton community was mostly comprised of diatoms (av. 73.5%) and blue green algae (av. 20.5%). *Rhizosolenia alata*, *R. stolterfothii*, *R. imbricata*, *Nitzschia longissima*, *Thalassiosira subtilis* etc were the predominant diatoms (Table 1). Blue green algae such as *Hapalosiphon hibernicus* (33200 cells L^{-1}) and *Trichodesmium erythraeum* (5333 cells L^{-1}) were at high density at station A1, with the former at the surface and the later at 10 m depth. In addition, dinoflagellates such as *Ceratium trichoceros*, *C. furca*, *Protoperdinium depressum* were also observed though in less numbers (Table 2). The phytoplankton cycle in the PB generally shows three seasonal peaks dominated by diatoms⁶. Eventhough monospecific blooms of *Rhizosolenia alata* and *R. imbricata* were occasionally reported in the Palk Bay, no information was available on any such symbiotic relationship. However, many reports have come out worldwide from the tropical and sub-tropical waters on the symbiosis of *Rhizosolenia/Richelia*⁵. Recently it has also been reported from the open waters of the Arabian Sea¹² and the Bay of Bengal¹³.

In fact, because of the symbiotic association, both host and the symbiont mutually benefited in such a manner that *R. intracellularis* enhances photosynthesis that will be useful to *R. hebetata* as it lacks sufficient cytoplasm and well developed chloroplasts¹⁴. On the other hand, the fragile filaments of *R. intracellularis* gets protection from the rigid frustules of *R. hebetata*¹⁵. Recent studies^{2,14} revealed that cytoplasm of the vegetative cells of *R. intracellularis* apparently lack gas vesicles that ultimately causes instability of the cells in the water column to live as free living plankton. Consequently, unattached filaments should not be viable in the water for a long time and therefore a host would be required for its further survival. The replication would therefore be after contact with the host cell and that is why *R. intracellularis* is found living as ecto- or endosymbiont in some diatoms¹⁶. There were many reports from the tropical waters mentioning the occurrence of extensive bloom of diatoms (mostly *Rhizosolenia* and *Hemiaulus*) containing endosymbiont *R. intracellularis*, which added significant new N to the oligotrophic waters². Culture experiments¹⁴ evidenced that there is direct excretion of fixed nitrogen into the surrounding medium of the

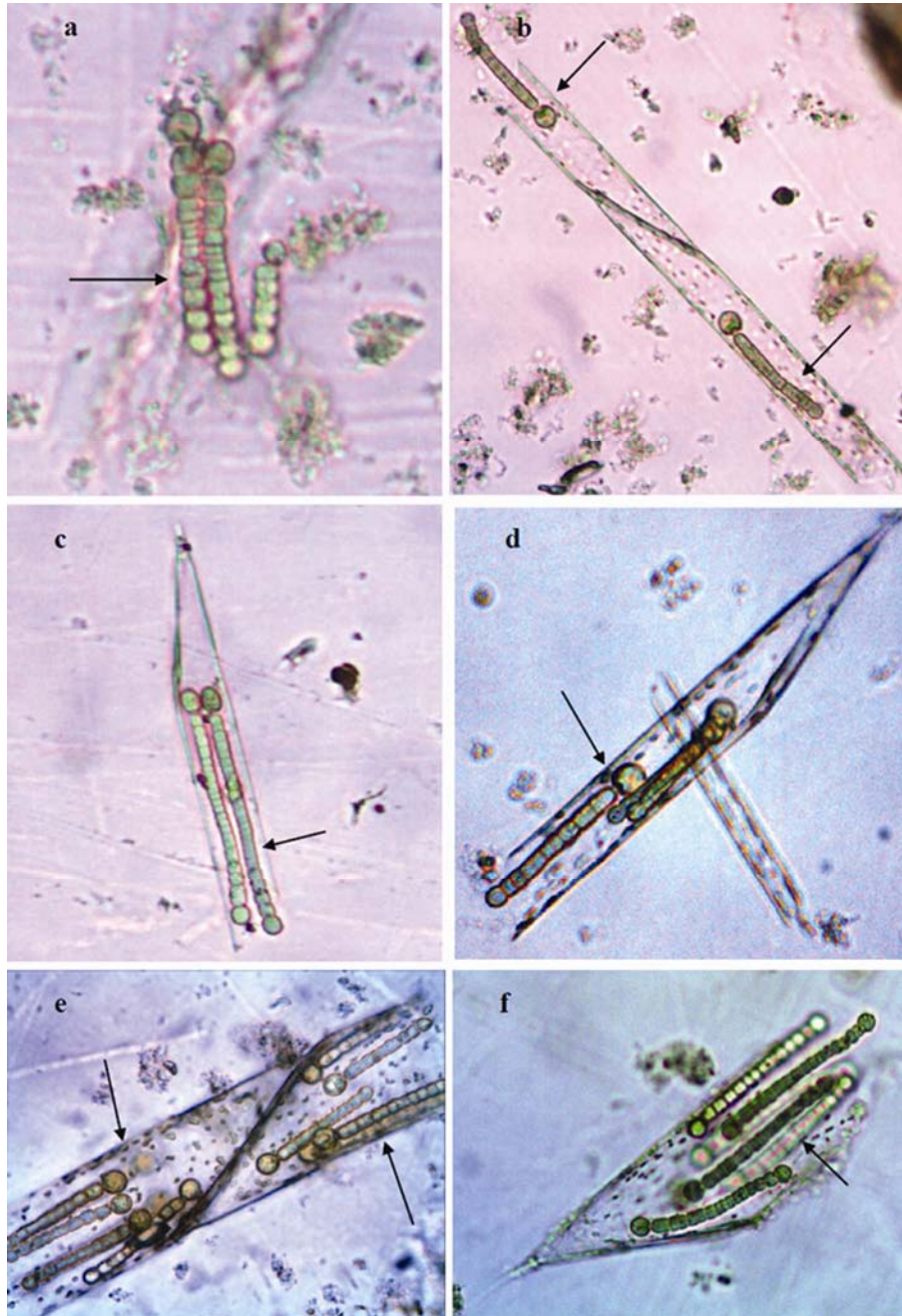


Plate 1— Photomicrographs of *Richelia intracellularis* as an endosymbiont in *Rhizosolenia hebetata*, a) free trichomes, b) one trichome, c) two trichomes, d) three trichomes, e) four & five trichomes, f) six trichomes in the frustules of *R. hebetata* (40 X magnification).

Table 1—Major water quality parameters at the stations where cyanobacteria-diatom symbiotic association observed

Parameters	St. A1			St. A2		
	surface	5 m	10 m	surface	5 m	10 m
Temperature (°C)	30.8	30.1	30.4	31.7	30.2	30.1
Salinity	31.0	31.0	32.1	28.1	30.1	30.1
Nitrate - NO ₃ (μM)	0.7	1.1	0.7	0.5	0.2	0.1
Chlorophyll <i>a</i> (mg m ⁻³)	0.6	0.4	0.8	1.8	1.1	0.9

Table 2—List of phytoplankton with abundance (cells L⁻¹)

	Station A1			Station A2		
	Surface	5 m	10 m	Surface	5 m	10 m
Diatoms						
<i>Skeletonema costatum</i>	-	34	-	-	-	250
<i>Thalassiosira subtilis</i>	-	-	-	1471	1576	1500
<i>Coscinodiscus</i> sp.	-	68	-	118	-	250
<i>Rhizosolenia robusta</i>	333	0	-	-	-	-
<i>R. imbricata</i>	333	0	200	59	72	-
<i>R. setigera</i>	-	34	200	59	0	-
<i>R. stolterfothii</i>	-	0	200	118	72	250
<i>R. hebetata</i>	-	68	200	-	-	-
<i>R. hebetata</i> + <i>R. intracellularis</i> *	120	260	-	-	140	250
<i>Bacteriastrum delicatulum</i>	333	-	-	59	144	-
<i>Bacteriastrum furcatum</i>	333	-	-	59	216	-
<i>Bacteriastrum hyalinum</i>	-	-	-	-	72	250
<i>Chaetoceros</i> sp.	-	34	-	353	725	750
<i>Guinardia flaccida</i>	-	-	200	118	144	500
<i>Climacodium frauenfeldianum</i>	333	-	0	-	-	-
<i>Biddulphia sinensis</i>	-	-	0	-	-	500
<i>Hemialus hauckii</i>	666	-	0	-	-	-
<i>Thalassionema nitzschioids</i>	-	-	0	-	144	-
<i>Licmophora</i> sp.	-	-	200	0	0	250
<i>Gyrosigma</i> sp.	-	-	400	59	72	0
<i>Pleurosigma elongatum</i>	-	-	0	59	576	150
<i>P. normanii</i>	-	-	0	353	72	0
<i>Navicula</i> sp.	-	-	200	0	0	250
<i>N. directa</i>	-	-	0	59	72	250
<i>N. distans</i>	-	-	0	0	288	250
<i>Amphiprora gigantea</i>	-	-	200	0	0	750
<i>Cylindrotheca closterium</i>	-	-	0	59	360	500
<i>Nitzschia</i> sp.	-	68	0	177	375	750
<i>N. longissima</i>	-	34	4400	4118	3720	4500
<i>Surirella</i> sp.	-	-	0	0	0	500
Dinoflagellates						
<i>Prorocentrum micans</i>	-	-	0	59	144	0
<i>P. gracile</i>	-	-	0	0	0	500
<i>Ceratium trichoceros</i>	333	102	200	0	0	0
<i>C. furca</i>	0	34	200	59	72	0
<i>Gonyaulax</i> sp.	0	68	0	0	72	0
<i>Pyrophacus</i> sp.	0	34	0	0	72	0
<i>Protoperidinium depressum</i>	0	0	200	0	0	250
Blue green algae						
<i>Trichodesmium erythraeum</i>	5333	748	200	59	72	0
<i>Nostoc</i> sp.	0	0	200	0	72	0
<i>Hapalosiphon hibernicus</i>	0	0	33200	0	0	0
<i>Richelia intracellularis</i>	80	0	0	0	0	0
Others	0	0	0	59	0	0

R.intracellularis and this would allow the immediate consumption of the *Rhizosolenia* cells. Based on the present observation, it could be stated that instead of common occurrence of *Rhizosolenia-Richelía* symbiosis in the open waters of tropical and sub-tropical seas, it can be survived in the shallow waters also if there is nitrate depletion exists. Even though the symbiotic association between the *Rhizosolenia-Richelía* did not reach bloom condition, its occurrence can be promoted an unusual food web in the Palk Bay.

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